experimental data into Ising-like statistical mechanical models to better understand the observed structural and energetic properties of the two proteins. Two variations of the Ising-like model were implemented: the Wato-Saito-Muñoz-Eaton (WSME) model, which can be enumerated exactly using efficient transfer matrix methods, and the Baker-Finkelstein (BF) model using a double-sequence approximation. Model parameters were optimized by simultaneously fitting the complete set of data for the whole protein as well as each helix independently to reflect what was observed through experiments. In order to give a more realistic representation of protein energy, various statistical residue-specific potential matrices were tested as the inter-residue contact energy in the model. We found that different statistical potentials varied in its success to simultaneously fit all the experimental data, however all the residue-specific matrices resulted in an improvement over considering only a single parameter for the contact energy. Both the WSME and BF models were able to reproduce the equilibrium unfolding data when analyzing the hth proteins as a whole, but the WSME model could not correctly predict the folding of only the helix when analyzed independently due to the assumptions of the model. On the other hand, the BF model was capable of reproducing the experimental data for both the whole protein and the independent helices.

3403-Pos Board B131

Towards a Test of the Aggregation Hypothesis in Huntington's Disease using β-Hairpin Enhancing Motifs

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Huntington's Disease (HD), one of ten polyglutamine (polyQ) repeat diseases, is a devastating disorder caused by expansion of a polyQ-encoding CAG repeat from 37 or more in exon1 of the huntingtin (htt) gene. Although HD brains contain polyQ aggregates, and polyQ aggregation rates *in vivo* and *in vitro* increase with repeat length, there is a continued debate about the role of amyloid-like aggregates in HD. Recently we reported that aggregation of chemically synthesized, short (repeat length ~ 22), simple polyQ sequences is greatly enhanced with the addition of unnatural amino acids that encourage β-hairpin formation in the aggregation nucleus. Here we ask whether β-hairpin encouraging mutations in a short polyQ version of the htt exon1 peptide also greatly enhance aggregation. We do this while confining our study to mutations that can be introduced during ribosomal synthesis. We show here that a short polyQ sequence containing (a) L-Pro-Gly instead of the previously described D-Pro-Gly and (b) a modified tryptophan zipper motif aggregates much faster than a simple polyQ sequence of similar length. This can be traced to a decrease in the critical nucleus for amyloid formation from a value of $n^* \approx 4$ for a simple, unbroken Q_{23} sequence to $n^* \approx 1$ for similar length polyQ containing β-hairpin motifs. At the same time, the morphologies, secondary structure structures, and bioactivities of the resulting fibrils from simple and exon1 mimic polyQ were essentially identical. Importantly, incorporating these motifs into short polyQ exon1 analogs produces rapid spontaneous aggregation rates comparable to exon1 peptides with long, disease associated polyQ repeat lengths. Expression of these exon1 analogs in cells now addresses whether even short polyQ htt exon1 can be toxic if its polyQ is redesigned to promote rapid aggregation.

3404-Pos Board B132

Salt Effects on Folding of a Helical Mini Protein Villin Headpiece Subdomain HP36 Studied by Generalized-Ensemble Simulations

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Additives dissolved in solvent are important factors that affect proteins' stability and/or folding. In this study we investigated effects of salt ions in solvent on folding events of a helical mini protein HP36. Addition of low concentrations of ions should alter electrostatic interactions among charged groups, so that populations for conformational substates of proteins should be changed. Here we compared two data sets of folding simulations of HP36 with explicit water solvent. For efficient sampling of conformational space of the protein, multicanonical replica-exchange method was adopted.

Results of the present analyses suggest that addition of ions reduces the number of nonnative, nonlocal salt bridges in the protein molecule at later stages of folding at room temperature. Especially, nonnative salt bridges between Glu5 and Arg15 and/or another between Asp4 and Lys30 have been kept in the near-native conformations in pure water. Because dehydration of the hydrophobic core of HP36 is completed only at the latest stage of folding where correct hydrophobic-core packing becomes formed, these salt bridges can prevent folding into the fully native structure of HP36 at room temperature.

3405-Pos Board B133

Simulation Model of Protein Transport and Stabilization by GroEL/ES

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In a previous communication (Linhananta et al., *Biophys. J.*, 2011, 100, 459), we reported results of a simulation model of a protein in solvents with protein-solvent contact energy parameter ε_{PS}, which represents osmolytes ($\varepsilon_{PS}>0$) and denaturants ($\varepsilon_{PS}<0$). Here a model of a three-helix-bundle (THB) protein in solvents is confined in a cylindrical cavity that mimics the GroEL/ES chaperone. The interior is characterized by the protein-wall energy, ε_{PW}, and solvent-wall energy, ε_{SW}. Simulations found a substantial increase in the folding temperature from $T^* = 4.2$ (scaled unit), for THB in vacuum, to $T^* > 6.0$ for confined THB in osmolytes. The optimum stabilization of the native state is $T^* = 6.6$, for THB in osmolytes with $\varepsilon_{PS} = 0.6$, confined by walls repulsive to THB ($\varepsilon_{PW} = 1.0$) and solvents ($\varepsilon_{SW} = 1.0$). Weight histogram analysis reveals an entropy-driven stabilization mechanism due to confinement and the osmolytes. The model is generalized to THB and solvents confined in two connected cylindrical segments. The bottom segment represents the GroEL/ES, with the interior sidewall characterized by the parameters ε_{PW} and ε_{SW}. The upper segment represents the exterior surrounding the GroEL/ES, with periodic boundary condition on the sidewall, where the protein and solvents can move through the channel connecting the two segments. For neutral solvents ($\varepsilon_{PS} = 0$) with a sidewall that is repulsive to solvents ($\varepsilon_{SW} > 0$) and attractive to the protein ($\varepsilon_{PW} < 0$), the THB protein preferentially distributes in the lower segment that represents the interior of the GroEL/ES. As the temperature increases and the protein denatures, there is an increase in the probability that the protein is found in the GroEL/ES. This highlights the roles of solvents and surface properties in the transport of unfolded proteins into the GroEL/ES.

3406-Pos Board B134

Non Local Interactions are Essential Elements of the Initiation and Guidance of the Folding Pathway of Proteins

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The rate of protein folding is determined by the rate of passage through the transition state, however major structural transition precede the TSE formation. We hypothesize that few non-local interactions are effective in the early phases of the folding transition prior to the cooperative transition. These interactions loosely stabilize few closed loops which form the folding noncontiguous nucleus, reduce the chain entropy and determine the course of the folding pathway (the ''loop hypothesis''). We study the order of formation of secondary structure elements and loop closure during the early phases of the folding of *E. coli* adenylate kinase (AK) by combination of rapid mixing methods and time resolved FRET spectroscopy. We find that at the initiation of folding of the AK molecule two closed loop structures in the CORE domain reach native end to end distance within a millisecond while a third loop (the N terminal loop) is closed on the microsecond time scale. Three representative CORE domain β-strands have non-native end to end distance during the first 15 ms and undergo slow change (3 sec) to native distance. Along the folding pathway of AK the fast closed N terminal loop is reopened and closed again. We conclude that non local interactions are essential factor at the early phases of the folding transition and that the folding of sub-domain elements is context dependent and should be studied in the whole molecule, *in situ*. .

3407-Pos Board B135

Computational Studies of the Formation of Peroxiredoxin Dimers

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The proteins in the ubiquitous peroxiredoxins (Prx) protein family play an important role in redox signaling and antioxidant defense. The biological functions of Prxs are closely related to the formation of their quaternary structures. To understand details of interactions within Prxs and their quaternary structures formations, the disassembly and unfolding processes of 1YEP (chains A and B) and 3DRN were studied as an example through molecular dynamics simulations. Hundreds of four-microsecond-long simulations using a Go-type model show that disassembly and unfolding processes are

correlated but disassembly occurs more readily. This indicates monomers form faster than the quaternary structures. Characteristics of monomer-monomer interfaces were observed between 1YEP chain A and chain B and BCP1 (3DRN) while the characteristic of dimer-dimer interface was seen between 1YEP chain B and chain C.

3408-Pos Board B136

Investigating Characteristics of Folding Cores from Analyses of Folding Mechaniams on Multi-Transition Proteins by Means of a Coarse-Grained Go Model

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Though it has become clear that small and fast folding proteins have funnel shaped energy landscape, we have not know explicitly what and when happens in folding process. The knowledge of precise picture in each transition in the folding process of multi state proteins should provide the hints to elucidate the detailed folding processes.

However, many hypotheses that try to explain the mechanisms of multi-state folding have been proposed (such as hypotheses regarding the packing of side chains after searching the protein's topology, those regarding a number of subdomains that form a distinct barrier, those regarding discrete two-dimensional and three-dimensional structure formation, and others) and we have not yet gained a precise understanding.

To understand the mechanisms of multi-state folding, thermodynamic contributions of each component of the system, such as a main-chain, a side-chain and water, should be specified. It can be accomplished by comparing the simulation results of some models which has different resolution such as Ca-Go model, full-atom Go model and all-atom with explicit water.

In the precedent study, as a first step, we showed that the difference in the shape of the free energy profiles could be interpreted by the number of the regions that folds cooperatively and degree of coupling of the regions by means of modified Ca-Go model.

In this study, we apply the same method with previous work to other proteins which are suggested to fold into the native structures through multiple transitions and affirm the number of the barrier and stability of the intermediate states. We also investigate that the characteristics of the regions to contribute to barrier constructions.

3409-Pos Board B137

Effects of Energetic Heterogeneity on Protein Folding Dynamics Across Many Non-Homologous Proteins

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Native structure based models (SBMs, ''Go-models'') are based on energy landscape theory and the principle of minimal frustration [1,2]. Using this framework strongly reduces computational demands and allows us to investigate the influence of the contact-energy weight onto the folding process for many different protein families. A large set (approx. 200) of non-homologous monomeric proteins sized from 50-150 amino acids are simulated on a coarse-grained level, representing each amino acid by a single bead. A fully automatized workflow implemented with the help of eSBMTools [3] allows to quantify typical folding properties like phi-values, folding free energy landscapes and transition state ensembles (TSEs) for the simulated proteins.

Conventional SBMs use sequence independent homogeneous energy weights for the native contact energy. We compare these ''vanilla'' models with sequence dependent heterogeneous ''flavored'' energy weights as introduced by Miyazawa and Jernigan [4]. Subsequently, the dynamics of forming TSEs are compared by the sequential formation of tertiary interfaces between secondary structure elements in vanilla and flavored simulations. We find that, despite the energetic dissimilarity, the sequential ordering is similar between both types of simulations and appears as a robust property. This suggests that protein folding dynamics are strongly influenced by the native protein topology.

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3410-Pos Board B138

Aromatic Amino Acids Promote Peptide Folding by Reducing Backbone Hydration

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The relation between the sequence of a protein and its tridimensional structure remains largely unknown. To better understand how single residues impact on the local protein structure, we studied peptides of sequence EGAAXAASS (X = Gly, Ile, Tyr, Trp) through comparison of molecular dynamics (MD) trajectories and NMR residual dipolar coupling (RDC) measurements. The RDC patterns of the peptide with X = Gly or Ile are rather flat, suggesting extended, unfolded peptides, while the contrasted patterns for peptides with X = Tyr or Trp suggest compact folded structures. The comparison shows that the formation of internal hydrogen bonds underlying helical-turns is key to reproduce experimental RDC values for the peptides containing aromatic residues. The simulations further reveal that the driving force leading to such helical-turn conformation arises from the lack of hydration of the peptide chain on either side of the bulky aromatic side chain, which can potentially act as a nucleation point initiating the folding process. These results provide a starting point to understand the amino acid code underlying the mechanism of protein folding.

3411-Pos Board B139

pH Dependent Conformational Change of Hepcidin and its Precursor Protein, Pro-Hepcidin

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Hepcidin plays a role in the regulation of iron homeostasis through its interaction with an iron transpoter protein, ferroportin, in the liver. Hepcidin consists of 25 amino acid residues and four intra-molecular disulfide bonds, which are absolutely required for its biological activity, not only for iron homeostasis, but also for anti-microbial activity. To investigate structure-function relationships, hepcidin was chemically synthesized. However, it is well known that the synthetic yield of hepcidin is quite low under the typical folding conditions. To overcome this issue and regulate the disulfide-coupled folding of hepcidin, we studied the conformation of reduced/denatured hepcidin by means of CD spectroscopy under a variety of conditions. In addition, recombinant pro-hepcidin was also prepared using *E. coli* expression system and its conformation investigated, since the disulfide-coupled folding of hepcidine proceeds via a precursor *in vivo*. The major problem of the disulfide-coupled folding of hepcidin is that it undergoes aggregation during its folding reaction. To solve this problem, several types of redox reagents and solvents were examined to improve the folding efficiency of hepcidin. However, all of the reagents resulted in quite low yields for the disulfide-coupled folding of hepcidin. Therefore, we estimated the pH of the folding solution used to regulate disulfide-coupled folding of hepcidin since the folding velocity and conformation of disulfide-containing peptide is significantly affected by the pH of the solution. Our experimental results show that reduced/denatured hepcidin is not stable around neutral pH's, although the native hepcidin is quite soluble in aqueous buffers at a neutral pH. Alkaline conditions were also not effective for the folding reaction. Therefore, we studied the conformation of hepcidin and pro-hepcidin under acidic conditions. The results will be discussed in this paper.

3412-Pos Board B140

Myosin UNC-45 Chaperone: The Role of its Domains in the Interaction with the Myosin Motor Domain

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The proper folding of many proteins can only be achieved by the interaction with molecular chaperones. The molecular chaperone UNC-45 is required for the folding of striated muscle myosin II, however, the precise mechanism by which it contributes to proper folding remains unclear. UNC-45 contains three domains: an N-terminal TPR domain known to bind to Hsp90, a central domain of unknown function, and a C-terminal UCS domain known to interact with the myosin head. Here we used fluorescence titrations methods, dynamic light scattering, and single-molecule atomic force microscopy (AFM) unfolding/refolding techniques to study the interactions of the UCS and central domains with the myosin motor domain. To study the effects of UNC-45, UCS and central domain binding to the myosin motor domain we used environmentally sensitive fluorescence as a reporter for the UNC-45 domain-myosin interaction. We found that the UCS domain and the central domain bind to distinct sites on the myosin motor domain. In order to test which UNC-45 domain has a chaperone activity we used two different methods: i) prevention of misfolding using single-molecule AFM, and ii) prevention of aggregation using dynamic light scattering manner. Using the first method we found that the UCS domain is sufficient to prevent misfolding of a titin mechanical